



IN THE CLAIMS:

Please cancel claims 23, 24, 26-34, 36-38 and 43, without prejudice.

Please amend the claims as follows:

Please amend claim 1, with the clean version provided below to read as follows:

1(Twice Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, comprising:

- a) a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron;
- b) a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;
- c) optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and
- d) a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter.

Please amend claim 18, with the clean version provided below to read as follows:

18(Twice Amended). A polynucleotide which cannot replicate in eukaryotic cells *in vivo* and which comprises contiguous nucleic acid sequences capable of being expressed to produce a gene product upon introduction of the polynucleotide into eukaryotic tissues *in vivo*, wherein the gene product either acts as an immunostimulant or as an antigen capable of generating an immune response, wherein the nucleic acid sequences encode:

- a) a spliced REV gene;
- b) a spliced human immunodeficiency virus (HIV) immunogenic epitope; and
- c) optionally, a cytokine or a T-cell recognition element.

Please amend claim 22, with the clean version provided below to read as follows:

22(Twice Amended). A polynucleotide comprising a first gene encoding an HIV gag, gag-protease, or env immunogenic epitope, the first gene containing a REV responsive element (RRE) or having been modified to contain an RRE, the first gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the first gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene encoding a REV gene product, wherein said polynucleotide is non-replicating in eukaryotic cells *in vivo*.

Please amend claim 24, with the clean version provided below to read as follows:

24(Twice Amended) A polynucleotide which is non-replicating in eukaryotic cells *in vivo* and induces anti-HIV neutralizing antibody, HIV specific T-cell immune responses, or protective immune responses upon introduction into vertebrate tissue, including human tissue *in vivo*, wherein the polynucleotide comprises a gene encoding a gene product selected from the group consisting of HIV gag, HIV gag-protease, and HIV env, the gene containing a REV responsive element (RRE), the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene, the second gene encoding a REV gene product.

Please amend claim 35, with the clean version provided below to read as follows:

35(Twice Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising:

- a) a eukaryotic transcriptional promoter;
- b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;
- c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;
- d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;

- e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes being selected from the group consisting of GM-CSF, IL-12, interferon, and a B7 protein; and,
- f) a transcription-termination signal following the last open reading frames.

Please amend claim 39, with the clean version provided below to read as follows:

39(Twice Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

- a) a eukaryotic transcription initiation signal;
- b) an HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;
- c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;
- d) a sequence encoding an ORF of a T-cell costimulatory element 3' to the IRES; and
- e) a transcription termination signal 3' to the translation stop codon of the T-cell costimulatory element.

Please amend claim 41, with the clean version provided below to read as follows:

41(Twice Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

- a) a eukaryotic transcription initiation signal;
- b) a first HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;
- c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the first HIV ORF;
- d) a second HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the second HIV gene ORF does not depend on availability of the HIV REV gene product; and
- e) a transcription termination signal 3' to the translation stop codon of the second HIV gene ORF.

Please amend claim 42, with the clean version provided below to read as follows:

42(Twice Amended). A composition comprising multiple polynucleotide expression vectors of claim 1, each polynucleotide expression vector, upon *in vivo* introduction into a mammalian cell, being non-replicating but being capable of inducing expression of more than a single cistron contained within the polynucleotide expression vector, the cistrons encoding antigens related to disease causing pathogens or tumors.

Please amend claim 44, with the clean version provided below to read as follows:

44(Twice Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, the polynucleotide comprising a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter, optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter, and a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter, wherein each of the first, second and optionally third cistrons encode a combination of any two to three of the following:

- 1) tPA-gp120MN;
- 2) gp160III^B/IRES/REVIII^B;
- 3) gp160III^B;
- 4) REVIII^B;
- 5) tat/REV/gp160;
- 6) REV/gp160;
- 7) gp160MN;
- 8) gp160 from clinically relevant primary HIV isolates;
- 9) nef, using the gene from clinically relevant strains;
- 10) gagIII^B;
- 11) tPA-gp120III^B;

12) gp160 with structural mutations including V3 loop substitutions from clinically relevant strains of HIV; several mutations on several constructs such as variable loop removal, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;

13) gp41 with provision of appropriate leader sequences, as in the tPA signal peptide leader sequence;

14) *gag*: similar to construct from #5 above, using the gene from clinically relevant strains;

15) *rev*: for gp160 and *gag* dicistronics;

16) B7 coding sequences;

17) GM-CSF sequences;

18) Interleukin sequences;

19) Tumor associated antigens;

20) Genes encoding antigens expressed by pathogens other than HIV, such as, but not limited to, influenza virus nucleoprotein, hemagglutinin, matrix, neuraminidase, and other antigenic proteins; herpes simplex virus genes; human papillomavirus genes; tuberculosis antigens; hepatitis A, B, or C virus antigens; and combinations of these and other antigens to form at least dicistronic constructs which may be combined with multiple other polycistronic constructs to provide a cocktail composition capable of raising immune responses against all of the represented pathogens or tumor antigens.

Please add new claim 45, as follows:

45(New). The polynucleotide construct selected from the group consisting of V1Jns-(tat/rev SD), V1Jns-gp160_{IIIB}/IRES/rev_{IIIB} (SD), V1Jns-gag-prt_{IIIB} (SD), V1Jns-gag-prt_{IIIB}, V1Jns-tPA, V1Jns-tPA-gp120_{MN}, V1J-SIV_{MAC251}p28 gag, V1J-SIV_{MAC251}nef, and V1Jns-tat/rev/env.